miR-29 regulation of B cell class switch recombination

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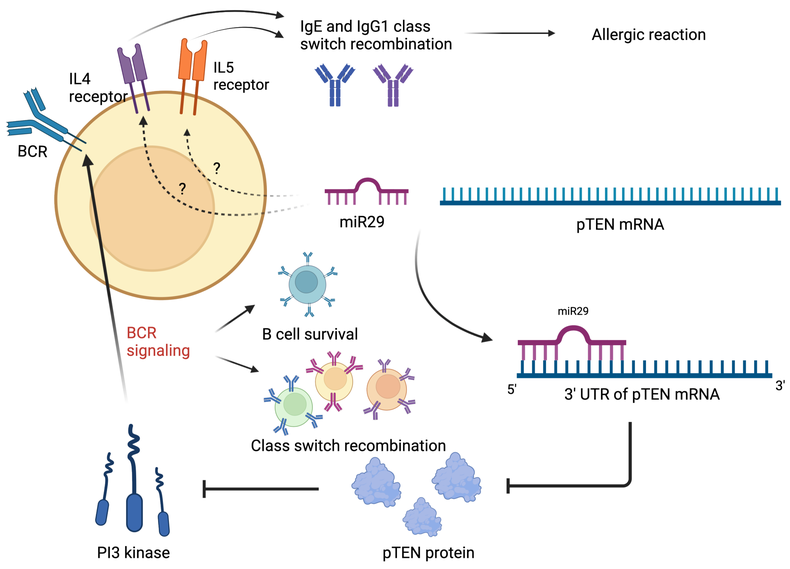
INTRODUCTION



miR29 micro RNA was shown to inhibit the translation of pTEN protein in B cells. pTEN is responsible for the negative regulation of the kinase PI3 which is associated with BCR (B cell receptor) signaling. BCR signaling promotes B cell survival and class switch recombination.

In a previous study miR29 was knocked out together with pTEN gene which, in theory, should have led to normal B cell development. However, although these mice did have mature B cells they also experienced inflammation and early death. Therefore we hypothesise that miR29 might be targeting cytokine receptors that control the developement of B cells that are responsible for allergic reaction and autoimmunity. During the target scan analysis of miR29 two such cytokine receptors (IL4 and IL5) were discovered. These receptors are responsible for B cell class switch recombination to IgE and IgG1 antibody producing B cells. Therefore we hypothesize that miR-29 mice B cells may be more predisposed to class switch compared to wild type mice.

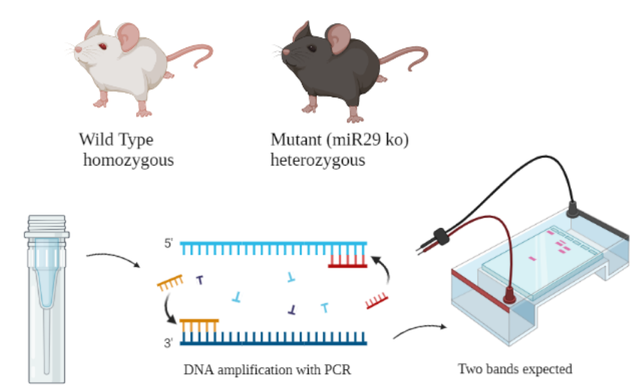
OBJECTIVE

Analyse the influence of miR29 on B cell ability to perform class switch recombination:

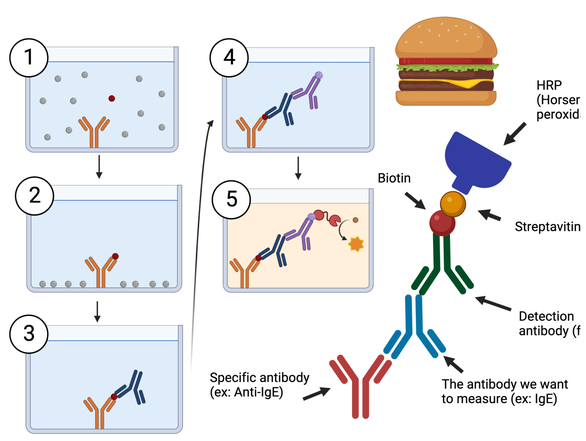
1) Confirm presence of mir29 genetic deletion in experimental mice.
2) Stimulate mir29-/- and wild type B cells in culture to class switch.
3) Measure and compare antibodies from culture and serum by flow cytometry and ELISA.

METHOD 1: Genotyping

Genotyping was used to confirm the genetics of our mice. Firstly, we extracted the DNA out of mouse tails, secondly, we amplified DNA by PCR, lastly, pass the amplified DNA through the gel-electrophoresis. This mouse genotype is heterozygous for the Mb1Cre allele, and this leads to a deletion of miR29 in B cells. Because the mice are heterozygous for Mb1cre one fragment of DNA is expected to be longer thus leading to two seperate bands on the gel.



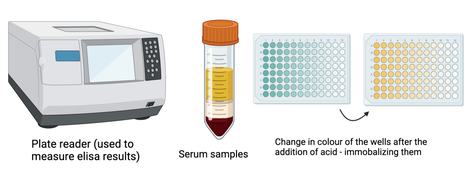
METHOD 2: ELISA



B cells were cultured under 3 different stimulation conditions to test class switch recombination. miR29-/- and wildtype derived B cells were exposed to either anti-CD40, anti-CD40 with IL-4, or anti-CD40 with IL-4 and IL-5 in order to stimulate IgE class switch recombination. Anti-CD40 was used to simulate T-cell activation of B-cells.

We then used ELISA (enzyme-linked immunosorbent assay) method to measure the concentration of antibodies in our samples. Our samples consisted of mouse serum samples (extracted from the blood) and culture supernatant samples. Serum samples were collected from the mice back in New York. Supernatant was gathered from cell cultures on day 2.

In our ELISAS we used anti-IgE capture and anti-IgE detection antibody to measure total IgE. We used anti-light chain and anti-IgG capture antibodies along with anti-IgG and anti-IgG3 detection antibodies to measure total IgG and IgG3.



RESULTS

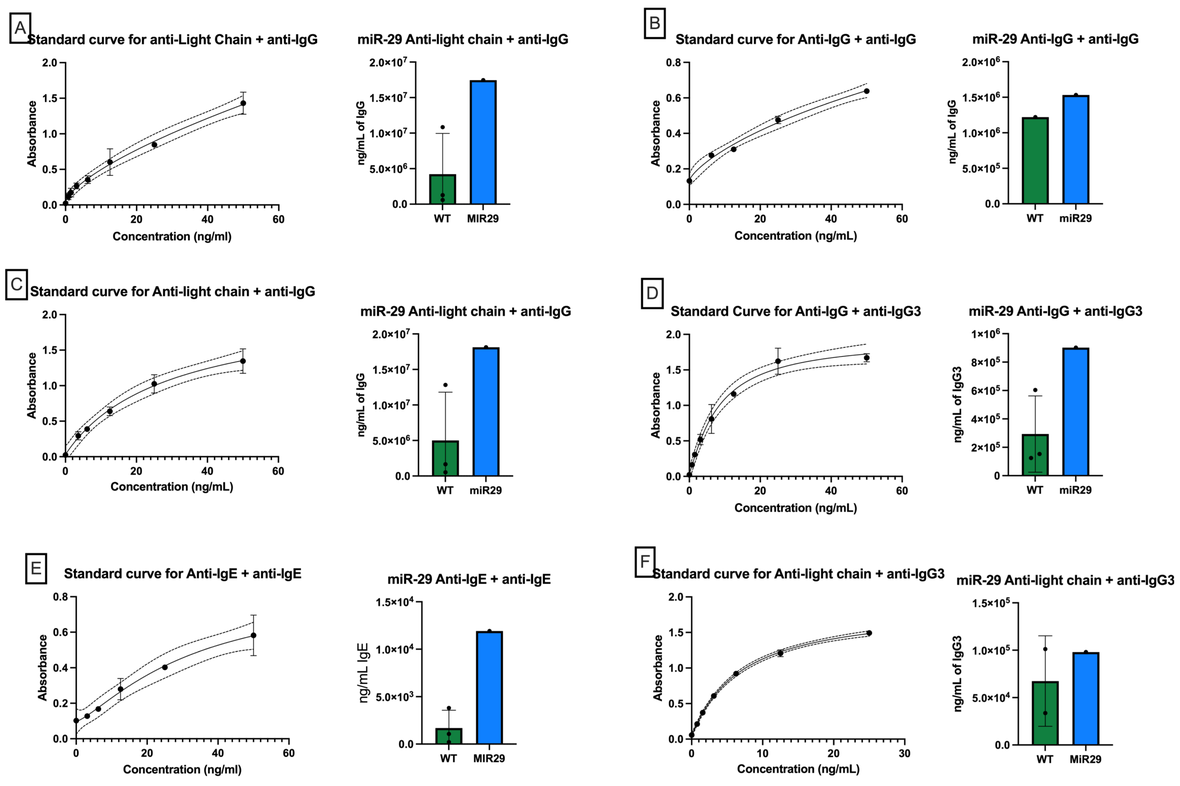


Figure 1. Graphs showing the output of the ELISAs performed in serum samples, including the standard curve and the concentration plot. For A and B we used anti-IgK/IgL (light chain) capture antibodies, and IgG detection antibodies. For C and F we used anti-IgK/IgL capture Abs, and IgG and IgG3 detection Abs respectively. For D, we used anti-IgG capture Abs, and IgG3 detection Abs, and for E anti-IgE capture and detection Abs.

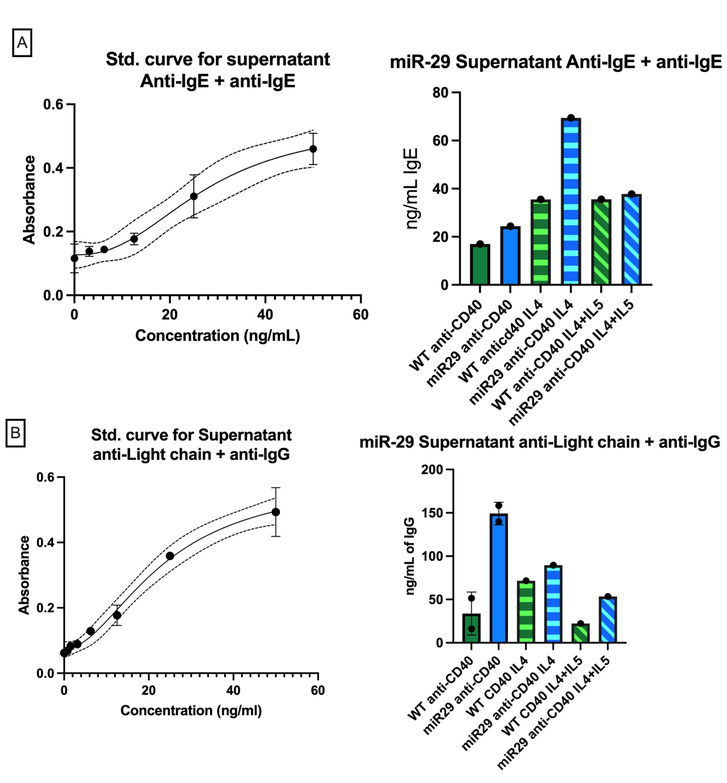


Figure 2. Graphs showing the standard curve and concentration plots for the ELISAs performed on day 2 miR-29 culture supernatant. In A we used anti-IgE capture Abs, and IgE detection Abs to measure total IgE. In B, we used anti-IgK/IgL capture Abs, and IgG detection Abs to measure total IgG.

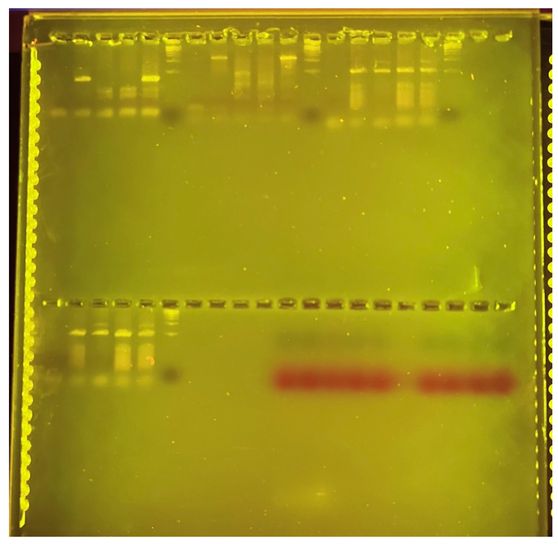


Figure 3. Photo of the gel electrophoresis we performed to evaluate the results of the PCR. Our wells are 13-17 on the first row, and 1-5 on the second one, which contain, in order: water, negative control, positive control, wild type and miR-29 knockout PCR samples.

CONCLUSIONS

The conclusions we can draw from our experiments are limited, because as the culture lost volume, the cells died before they class-switched and therefore, we couldn't observe their antibody production and functionality in full. Flow cytometry was planned in order to measure cell surface antibodies and intracellular antibodies, however due to the drying up of the cell culture we were unable to gather significant results. The supernatant ELISA outputs show a variable increase in IgE and IgG Ab production in the knockout compared to the wild type (Fig. 2). However, our sample size would need to be larger in order to perform the necessary statistical tests to make any definitive conclusions. As for the serum ELISAs, we observe a clear positive difference in IgE production in the knockout compared to the wildtype (Fig. 1 E), which fits the hypothesis, but the sample size would require further experimentation. In the IgG ELISAs, we notice a consistent increase in the knockout compared to the wildtype (Fig. 1 A, B, C). The same thing is apparent in the IgG3 detection wells (Fig. 1 D, F), but there is a distinction in the performance of the capture antibodies. There seems to be an order of magnitude of difference between the concentration detected by the capture Abs, the anti-light chain being better for IgG and anti-IgG for IgG3. Overall, our genotypes are correct given the results of the electrophoresis, however, we would need to repeat the experiment in order to draw proper conclusions.